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SHORTER ARTICLES AND DISCUSSION

THE EFFECT OF YEAST ON THE UTILIZATION OF FOOD BY WHITE MICE.[1]

IN the present paper the question of the effect of the so-called vitamines on basal metabolism is considered and a procedure is indicated whereby it is believed more conclusive data may be obtained on the question. Preliminary experiments are described illustrating the method.

Several years ago Hopkins[2] in a carefully carried out experiment investigated the effect of a small addendum of milk to a diet of purified food stuffs. In brief his method was to feed in pairs two sets of young rats of the same origin, weight, etc., on a basal vitamine-free food and to one set give a small addendum of milk and determine the food intake and growth increment. By comparing the energy consumption and growth increments of the two sets of animals and by comparing these factors on the same set of animals after reversing the diets he was able to show "that the small milk addendum reduced the food consumption for a given weight increment to one half or less." In other words the vitamine increased greatly the animal's power to utilize its food in the production of growth. And as he showed that this was not due to difference in absorption from the intestines, the vitamine must have an effect on some factor involved in basal metabolism.

A number of the criticisms that could be made of Hopkins' method have been discussed frankly by him and there is left little or no doubt concerning his conclusions. They have, however, such an important bearing on problems in nutrition that it seems very desirable that the question be investigated from all angles[3] and particularly with other animals,[4] and with vitamines from different sources. In this connection, it is believed that the procedure to be described later by the writer gives more direct evidence and hence is more free from criticism.

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[1] Contribution from the Department of Pharmacology, Harvard Medical School.

[2] *J. Physiol.*, 1912, 44, 425; see also *Biochem, J.*, 1913, 7, 97.

[3] Recently Eddy (*J. Biol. Chem.*, 1920, XXXIV; see also *Am. J. Dis. Children*, 1917, 14, 189), has reported cases of marasmic infants which showed an increase in the utilization of food, when given vitamine extracts prepared from beans.

[4] In another connection the writer has found that mice and rats react differently with certain diets. The question is still open whether or not this difference is qualitative or quantitative.

In order to eliminate the question of variability of individual animals from the point of view of efficiency as energy transformers, it appeared to the writer that a better procedure than that used by Hopkins would be to feed a number of animals a basal diet plus such an amount of vitamine-containing material as to keep the animal in weight equilibrium over a period of time. The amount of vitamine would vary with the individual, and would need to be determined in each case. When the animals had been maintained in weight equilibrium over a number of days and the food consumption noted, they could then be fed the same daily ration of a diet containing the same number of calories and having the same composition with the exception of a larger vitamine content and the weight noted. As the greater vitamine content would stimulate the appetite (directly or indirectly), there would be no difficulty about the animals eating the same amount of food as they had eaten in the first stage of the experiment and they would receive the same number of calories and a food with the same gross composition within very narrow limits.

Due to lack of time available for this work, it was impossible, in the preliminary experiment to be described, to bring each animal as near weight equilibrium as was wished, and as it is felt very certain can be done. The average, however, for twelve animals is close and if the results are considered from the statistical point of view, they give further very convincing evidence that the vitamines increases the efficiency of the body in the utilization of the food. The error due to temperature variation it is believed is not large, but this, of course, should have been eliminated. There appears no very easy way of eliminating the error due to greater activity of the animals in the second stage of the experiment. This error may be considerable for there is no question that the animals were markedly more active when given the limited diet with greater vitamine content. The direction of this error makes the results all the more convincing.

EXPERIMENTAL

Preparation of Diets.—The diets were prepared at the beginning of the experiment from the same stock of material and placed in stoppered containers in the ice box. The composition follows:

	Diet 401	Diet 403	Diet 405
Casein	17.5%	16.5%	15.5%
Starch	49.5%	48.5%	47.5%
Yeast	1.0%	3.0%	5.0%
Lard	18.0%	18.0%	18.0%
Butter	9.0%	9.0%	9.0%
Salts	5.0%	5.0%	5.0%

The yeast contained approximately 0.46 per cent. fat, 46.5 per cent. protein and 38.0 per cent. carbohydrate (32.26 "carbohydrate" plus 5.8 crude fiber). Omitting the negligible quantity of salts the gross composition of the diets was as follows:

	Diet 401	Diet 403	Diet 405
Protein	17.96%	17.88%	17.8 %
Carbohydrate	49.88[5]	49.64[6]	49.4[7]
Fat	27.05%	27.15%	27.25%
Salts	5. %	5. %	5. %

[5] Includes 0.06 per cent. crude fiber.

[6] Includes 0.12 per cent. crude fiber.

[7] Includes 0.3 per cent. crude fiber.

The diets richer in vitamine had slightly lower calorific value.

The *casein* was prepared from the 40-mesh commercial product by shaking up for several hours successively with two portions of 50 per cent. alcohol and one of 95 per cent. alcohol and drying in warm air.

The *starch* was prepared from commercial cooking starch (corn) by the same procedure as for casein.

The *butter* used was the clear fat obtained by decanting the melted butter through a dry filter.

The salt mixture was that described by Osborne and Mendel.[8]

The mice were kept in wire mesh cages under which were pieces of glazed paper to catch feces and wasted food. The food was placed in small salve jars having aluminum covers in which ½ inch holes had been stamped. The jars were placed in 5-inch glass crystallizing dishes during the second stage of the experiment so that any waste food could later be found by the mice and eaten. Almost never in the second stage of the experiment was any food found under the cage. In all cases the waste was easily separated from the feces and account taken of the amount.

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[8] *J. Biol. Chem.*, 1919, 37, 572.

Sixteen mice were fed ten days on a complete diet and then nine days on a diet free of vitamines and then transferred to diet 401 (unlimited amount). After one day on the later intake records were begun.[9] Of the 16 mice started two grew nearly normally on 1 per cent. yeast,[10] one became sick and died, another declined very rapidly. These four were not considered in the experiment. The records of the remaining twelve are shown in the table. They were of varying size and represented somewhat different ages so that the results can hardly be accounted for by the action of the normal intermittent growth impulse.

PERIOD 1. DIET 401 (1 PER CENT. YEAST), UNLIMITED AMOUNT

Animal	Weights			Total Intake	Total Days	Average Daily Intake
	Beginning.	End	Change			
1.......	15.7 gr.	16.62 gr.	+0.92	27.15 gr.	13	2.09 gr.
2.......	11.2	10.49	−0.71	13.61	10	1.36
3.......	14.6	15.31	+0.71	24.75	13	1.9
4.......	11.1	10.45	−0.65	16.53	13	1.27
7.......	12.4	13.0	+0.60	19.83	13	1.52
8.......	14.0	14.97	+0.97	21.19	13	1.63
9.......	15.4	13.68	−1.72	19.39	13	1.49
10.......	14.8	13.71	−1.09	18.25	13	1.4
12.......	14.8	14.69	−0.11	21.91	13	1.68
13.......	18.5	17.05	−1.45	27.85	13	2.14
14.......	10.2	9.12	−1.08	13.77	13	1.06
17.......	15.9	16.58	+0.68	19.97	10	1.997
	168.6	165.67	−2.93	244.20	150	

For 150 mouse days 12 mice ate an average of 1.628 grs. per mouse per day and lost 2.93 grs. or 1.74 per cent. For maintenance, then, they needed slightly more than 1.628 grs. average per day.

Figured from Period No. 1 the mice in the 187 mouse days should have required a little more than 187 × 1.628 grs. and gained 14.36 grs. or 8.68 per cent.

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[9] It would be well to increase this period somewhat.

[10] In general mice require 5 per cent. of yeast in the diet for normal growth; some make substantial gains on 3 per cent. and a very small per cent. require less. The individual variation for rats is, in our experience, considerable though not as great as for mice. The average requirement, too, for mice is markedly more than for rats, the ratio being roughly five for mice to three for rats expressed in per cent. in the diet.

PERIOD NO. 2. DIET 403 AND (OR) 405 (LIMITED)

Animals	Beginnings	End	Change	Total Intake	Total Days	Fed Daily, Grs.
1.......	16.62 gr.	18.74 gr.	+ 2.12	32.64 gr.	16	2.04
2.......	10.49	11.8	+ 1.31	10.95	8	1.4
3.......	15.31	17.03	+ 1.72	30.40	16	1.9
4.......	10.45	11.83	+ 1.38	18.72	16	1.17
7.......	13.00	14.51	+ 1.51	24.00	16	1.5
8.......	14.97	14.05	– 0.92	24.00	16	1.5
9.......	13.68	14.45	+ 0.77	24.00	16	1.5
10.......	13.71	15.25	+ 1.54	24.00	16	1.5
12.......	14.69	14.65	– 0.04	25.6	16	1.6
13.......	17.05	18.84	+ 1.79	35.36	16	2.21
14.......	9.12	9.69	+ 0.57	17.00	16	1.06
17.......	16.58	19.21	+ 2.63	38.00	19	2.00
	165.67	180.05	+14.38	304.67	187	

In order to make more easily comparable the weight changes with food intake in the two periods, table No. 3 is presented.

Animal	Period No. 1			PeriodNo 2		
	Weight Change	Average Daily Food Consumption	Days	Weight Change	Average Daily Food Consumption	Days
1.......	+0.92 gr.	2.09 gr.	13	+2.12 gr.	2.04 gr.	16
2.......	–0.71	1.36	10	+1.31	1.4	8
3.......	+0.71	1.9	13	+1.72	1.9	16
4.......	–0.65	1.27	13	+1.38	1.17	16
7.......	+0.60	1.52	13	+1.51	1.5	16
8.......	+0.97	1.63	13	–0.94	1.5	16
9.......	–1.72	1.49	13	+0.77	1.5	16
10.......	–1.09	1.4	13	+1.54	1.5	16
12.......	–0.11	1.68	13	–0.04	1.6	16
13.......	–1.45	2.14	13	+1.79	2.21	16
14.......	–1.08	1.06	13	+0.57	1.06	16
17.......	+0.68	1.997	10	+2.63	2.00	19

The energy content of the excreta during the two periods was not determined, but Hopkins[11] and Drummond[12] have shown that the energy content of the excreta of rats when fed adequate foods is substantially the same as when fed vitamine-free foods.

The writer regrets the inability to repeat the work with a larger number of animals. It would be desirable to use a vitamine extract, control the temperature of the animal room, get the animals more closely in weight equilibrium by using a vitamine percentage determined by the individual mouse, and to have periods 1 and 2 run over the same number of days. Among the animals there should be included several adults which had been brought to condition of underweight in the preliminary period with vitamine-free foods. This would answer any possible question concerning the effect of the intermittent growth impulse. It is hoped that others may be able to carry on this work.

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[11] *J. Physiol.*, 1912, 44, 440.

[12] *Biochem. J.*, 12, 25.

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INTERFERENCE IN PRIMULA SINENSIS

OUTSIDE of *Drosophila*, the only data bearing on the question of interference of crossing over are those which I reported in a paper on linkage in *Primula sinensis*.[1] The phenomenon of interference—our knowledge of which in *Drosophila* dates from crosses made by Sturtevant and analyses made by Muller in 1912—consists of the fact that the occurrence of a crossing over in one region of a chromosome reduces the chances for the occurrence, in that cell, of another crossing over in a different region of the same chromosome; thus there is a smaller number of double crossovers than would otherwise be expected. The amount of interference is expressed by Muller's index called "coincidence," which is the ratio of the proportion of double crossovers actually observed in the experiment to the proportion of double crossovers which would have been expected to occur on the assumption that crossings-over in the two regions were independent of each other; the latter, or "expected" proportion of double crossovers is obtained by simply multiplying together the proportion of crossovers in one region by the proportion of crossovers in the other region. As I stated in my paper on *Primula*, a calculation based upon my total results could not be sufficiently reliable to decide the question of whether or not interference existed in *Primula*. This was on account of an uncertainty in the classification; I now find, however, that a calculation based upon a selected group of the plants, in which the uncertainty does not exist, is sufficient to decide the question in the affirmative—contrary to my earlier conclusion.

Three pairs of genes were involved in the *Primula* crosses—

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[1] Altenburg, E., 1916, Linkage in *Primula sinensis*. *Genetics*, 1: 354–366.